

In re Application of:
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Application No.: 09/910,706
Filed: July 20, 2001
Page 2

PATENT
Attorney Docket No.: JHU1520-2

AMENDMENTS

IN THE SPECIFICATION:

Please enter the following as a replacement for the paragraph at page 1, lines 4-6:

--RELATED APPLICATIONS

This application claims the priority under 35 U.S.C. §120 of U.S. Application No. 09/042,428, filed March 13, 1998, now issued U.S. Patent No. 6,294,355, which claims priority under 35 U.S.C. §119 of U.S. Provisional Application No. 60/036,553, filed March 14, 1997, which is pending as of the filing date of the present application, and which is both of which are incorporated herein by reference in their entirety.

GOVERNMENTAL SUPPORT--

Please enter the following replacement paragraph at page 3, lines 17-19:

--In a related embodiment, the invention includes polypeptides as described above, but which further exhibit an ability to selectively bind to a synaptic membrane protein having a C-terminal peptide region selected from the group consisting of SSSL (SEQ ID NO: 11) and SSTL (SEQ ID NO: 10).--

Please enter the following replacement paragraph at page 4, lines 3-7:

--In a particular embodiment, the binding protein in the assay method is a metabotropic glutamate receptor polypeptide which includes a sequence selected from the group consisting of SSSL (SEQ ID NO: 11) and SSTL (SEQ ID NO: 10). In another particular embodiment, the binding protein is an mGluR linked to phosphoinositidase C. In yet another embodiment, mGluR is expressed in cells, and binding between the receptor and the binding protein is measured by measuring phosphoinositidase C activity in cells.--

Please enter the following replacement paragraph at page 10, lines 1-6:

--Further characterization of selected clones is carried out by insertion of the isolated coding regions into vectors for expression in an appropriate expression system, such as any one or more of the systems as described in Example 3, or in other appropriate systems known in the art. Translated products are then isolated, such as by the methods described in Example 4, and are tested for the ability to bind to specific target proteins in the CNS and binding specificity for a particular peptide binding sequence, such as the sequence SSTL (SEQ ID NO: 10) or SSSL (SEQ ID NO: 11), as discussed in Section III.C., below.--

Please enter the following replacement paragraph at page 10, lines 11-21:

--Thus, looking at the N-terminal region of the polypeptides shown in FIG. 2, it is apparent that the first 30 amino acids are invariant among the three sequences. However, positions 31-34 differ. The rat sequence is AVTV, while the human and mouse proteins share the sequence GHRF. From this variation, it is possible to construct polypeptides in which positions 31-34 have the variable sequences: A/G V/H T/R V/F. Further regions of variability are apparent from inspection of the aligned sequences. Certain regions of the rat Homer protein have been identified as significant in the context of its function. For example, the PDZ-like domain GLGF (SEQ ID NO: 14) sequence and preceding arginine at positions 87-90 and 81, respectively, may form a "binding pocket", based on the known binding pocket of the synaptic binding protein PSD95 (Komau, et al., 1995). In accordance with the foregoing guidelines concerning substitution, this region is invariant among the three exemplified synaptic activation proteins and should therefore be conserved in any sequences deduced from these proteins.--

Please enter the following replacement paragraph at page 16, line 32 to page 17, line 8:

-- Metabotropic glutamate receptors uniquely possess long cytoplasmic C-terminal tails that are 67% identical over the last 55 amino acids and terminate in similar sequences; --RDYTQSSSSL

(SEQ ID NO: 9) and --RDYKQSSSTL (SEQ ID NO: 5), respectively (FIGS. 9A-9E). To measure the binding interaction, mGluR5 and mGluR1 α were expressed in HEK-293 cells. Cell extracts were mixed with bead-linked GST-Homer and were then eluted with SDS loading buffer. Both transiently expressed full length mGluR1 α and mGluR5 bind the rat Homer fusion protein, as shown in FIGS. 10A and 10D. When the C-terminal 4 amino acids of mGluR5 were deleted, binding of the mGluR5 to Homer was reduced by greater than 70% (FIG. 10D, lanes 3 and 4). Comparison of the C-terminal sequences of other metabotropic glutamate receptors indicates that mGluR2 and mGluR3 receptors share a similar C-terminal --TSSL (FIG. 8), although they diverge from mGluR1 α and mGluR5 outside this region. Neither mGluR2 nor mGluR4 bind Homer protein (FIGS. 10B, 10C). An unrelated protein known to possess the C-terminal TSSL (RSK1) (SEQ ID NO: 15) was also tested for binding, but this protein did not bind Homer. Based on these data, it is believed that the final 4 amino acids are important, but not sufficient for binding.--

Please enter the following replacement paragraph at page 17, lines 14-19:

--The effect of deletion mutations of the Homer protein on its binding to mGluR5 was examined by measuring binding of the full length Homer-GST fusion protein to myc-tagged mGluR5 C-terminal 195 aa fragment expressed from HEK-293 cells. Similarly, deletion constructs lacking the C-terminal 55 amino acids also bound mGluR5. By contrast, deletion of the N-terminal 108 amino acids of the Homer protein, which includes the GLGF (SEQ ID NO: 14) sequence, abolished binding to mGluR5. These observations indicate a role for the GLGF (SEQ ID NO: 14) region in binding to the C-terminal sequence of mGluR5.--